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LIPIDS OF *Oenothera* SEEDS FROM DIFFERENT HABITATS. 1.

S. G. Yunusova,[1] M. S. Yunusov,[1] A. R. Karimova,[1] V. F. Mironov,[2] S. G. Minzanova,[2] A. I. Konovalov,[2] Yu. Ya. Efremov,[2] O. N. Denisenko,[1] and E. V. Chernova[1]

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The composition of lipids and fatty acids from seeds of Oenothera biennis *growing in the RF near Kazan′ was determined. The content of γ-linolenic acid in all groups of acyl-containing lipids in the oil to be 4.4%.*

Key words: *Oenothera biennis*, lipids, fatty acids, γ-linolenic acid.

Evening primrose (*Oenothera biennis*, Onagraceae) is an important source of oil used as a biologically active additive. It has a wide spectrum of pharmacological activity but is most often used for cardiovascular diseases because it has a distinct antisclerotic and cardioprotective action [1].

A characteristic feature of the oil of this plant is the presence in it of γ-linolenic acid (18:3, Δ6,9,12; ω-6) (GLA) that can be synthesized only from linoleic acid (18:2, Δ9,12; ω-6). γ-Linolenic acid is the first intermediate in the series of conversions of essential linoleic acid into arachidonic acid (20:4, Δ5,8,11,14; ω-6) in humans.

According to the literature, about 30 g of vegetable oil is required per day in the diet. Ideally this should consist of saturated (10 g), monoene (10-15 g), and polyene (8-10 g) fatty acids. The optimal ratio for ω-6 and ω-3 polyene acids is 4:1. It is thought that one of the best oils for the diet with respect to the ratio of fatty acids is evening primrose [2].

America and Canada are the world's producers of evening primrose seeds. The lipid composition of *O. biennis* growing in these countries is rather well studied [3, 4]. However, it is known that the composition of natural compounds, including lipids, depends on the environmental conditions, in particular, the habitat.

Despite the fact that this plant is easily cultivated, it has not been grown in Russia in commercial quantities and the corresponding oil is not produced.

The goal of our work was to determine the lipid composition in various primrose species from different habitats and the content of γ-linolenic acid.

We investigated seeds of evening primrose (*O. biennis* L., Onagraceae) growing in the RF in the Republic of Tatarstan.

Certain properties of the seeds and oil were determined beforehand by standard methods. These were moisture (7.2%), oil content (19.4%) of seeds, and acid number (1.6 mg KOH). According to these data, primrose seeds can be considered moderately oily with a low acid number.

Neutral lipids (NL) were extracted from ground air-dried seeds by extraction with hexane. Column chromatography (CC) over silica gel was used in the initial stages to study the NL composition. However, prolonged contact of the oil with silica gel during CC separation reduced the content of triacylglycerides (TAG) and led to the accumulation of free fatty acids (FFA) and other hydrolysis products, diacyl- and monoacylglycerides (DAG and MAG). We observed this phenomenon before during a study of *Aconitum septentrionale* seeds [5]. Therefore, total NL were subsequently separated using preparative TLC (PTLC) on silica gel. Thus, we isolated and identified six classes of compounds. The lipid composition of *O. biennis* seeds is given below.

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1) Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Sciences, 450054, Ufa, pr. Oktyabrya, 71, fax (347) 235 60 66, e-mail: msyunusov@anrb.ru; 2) A. E. Arbuzov Institute or Organic and Physical Chemistry, Kazan′ Scientific Center, Russian Academy of Sciences, 420088, Kazan′, ul. Akad. Arbuzova, 8, fax (8432) 75 22 53, e-mail: lsk@iopc.knc.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 430-433, September-October, 2007. Original article submitted September 3, 2007.

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Lipid class	*Content, %*
Neutral lipids	17.2[a]
Triterpene esters (TE)	4.2[b]
Triacylglycerides	90.1[b]
Free fatty acids	1.3[b]
Diacylglycerides	2.0[b]
Sterols	0.6[b]
Monoacylglycerides	1.8[b]
Glycolipids	0.2[a]
Monogalactosyldiacylglycerides (MGDG)	22.3[c]
Sterylglycosides (SG)	23.6[c]
Digalactosyldiacylglycerides (DGDG)	54.1[c]
Phospholipids	$2.2 \cdot 10^{-3}$[a]
Cardiolipin (CL)	3.6[d]
Phosphatidylglycerine (PG)	10.0[d]
Phosphatidylethanolamine (PE)	2.0[d]
Phosphatidylcholine (PC)	39.9[d]
Phosphatidylinositol (PI)	22.9[d]
Lyso-phosphatidylcholine (lyso-PC)	5.0[d]
Phosphatidic acid (PA)	16.6[d]

[a]Of mass of air-dried raw material; [b]of NL mass; [c]of GL mass; [d]of PL mass.

The principal class of NL (90%) was triacylglycerides (TAG). The fraction of FFA was 1.3%.

A study of NL from seed oil of *O. biennis* growing in Tashkent Botanical Garden isolated and identified oxidized lipids as triacylglycerides containing epoxy- and hydroxy acids in addition to the aforementioned classes of lipids [6].

Mild alkaline hydrolysis isolated fatty acids (FA) from the acyl-containing NL classes. These were identified as methyl esters by GC (Table 1). According to the results, primrose oil can be considered to be highly unsaturated (up to 97% unsaturated FA) with a significant content of ω-6 linoleic (18:2) acid (up to 91.0% in TAG). Unsaturated FA were present in significant quantities in TE, FFA, and MAG, which agrees with the literature and our previous work on lipids from other plant species [5, 7]. γ-Linolenic (γ-18:3) acid was present in all NL classes. Its content was from 1.1% (FFA) to 1.8% (TE and DAG). However, it occurred in greater amounts (4.4%) in the oil itself (in NL). This is possibly due to the decompsition of γ-linolenic acid during separation of the lipids. Despite the fact that seed oil from primrose growing in Tatarstan is practically the same as that reported in the literature [3] with respect to total content of saturated monoene and polyene acids, the content of γ-linolenic acid in it is less than in the samples studied earlier.

The unsaponifiable components of the seed oil were sterols (0.6%) and tocopherols. The latter could not be isolated. Sterols were present in the oil in both the free and bound state as esters mixed with TE (esters of triterpene compounds). The sets of sterols in the free and bound state as determined by GC—MS were practically identical. We identified β-sito-, stigma-, and campesterol, the main component of which was β-sitosterol. The main component of the triterpene alcohols was lupeol. Carotinoids were not observed in primrose seed oil.

GC analysis of unsaponifiable components of lipids of seed oil from *O. biennis* growing in England identified in the 4-demethylsterols (sterols) campesterol and β-sitosterol; in 4-methylsterols, obtusifoliol, gramisterol, and citrostadienol [4]. These data were confirmed using mass spectrometry [6]. Only β-sitosterol and lupeol were identified in the lipophilic components of *Oenotere lamarckiana* [8].

Pulp remaining after NL isolation was extracted with $CHCl_3$:CH_3OH in order to isolate and identify polar lipids. The resulting extract contained total glyco- (GL) and phospholipids (PL). They were separated using PTLC.

GL made up 0.2% by weight of the air-dried (a-d) seeds. Three fractions were isolated from the GL using CC and PTLC. These were identified by TLC by comparison with model compounds and based on qualitative reactions and chemical transformations as monogalactosyldiacylglycerides (MGDG), sterylglycosides (SG), and digalactosyldiacylglycerides (DGDG). The last compounds had the highest content.

SG were not hydrolyzed by mild alkaline hydrolysis. The products of acid hydrolysis contained a component with TLC mobility corresponding to sterols that gave a positive qualitative reaction for this class of compounds.

TABLE 1. Fatty-Acid Composition of Acyl-Containing Classes of Neutral and Polar Lipids from *Oenothera biennis* L. Seeds, %

Acid	ΣNL	TE	TAG	FFA	DAG	MAG	ΣGL	ΣPL
12:0	Tr.	Tr.	0.9	0.6	0.2	6.3	Tr.	Tr.
14:0	0.1	Tr.	Tr.	0.2	0.2	12.6	Tr.	Tr.
16:0	5.9	13.4	1.7	9.9	4.2	15.9	20.0	17.3
18:0	0.2	4.5	Tr.	0.5	0.6	14.0	11.0	25.0
18:1	5.0	6.6	5.2	7.1	7.1	7.5	11.0	16.7
18:2	84.4	73.7	91.0	80.6	85.9	42.1	46.9	28.3
γ-18:3	4.4	1.8	1.2	1.1	1.8	1.6	5.0	7.0
α-18:3	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	2.3	2.9
21:0	-	-	-	-	-	-	Tr.	1.8
22:0	-	-	-	-	-	-	3.8	1.0
Σsat. FA	6.2	17.9	2.6	11.2	5.2	48.8	34.8	45.1
Σunsat. FA	93.8	82.1	97.4	88.8	94.8	51.2	65.2	54.9

In addition to the aforementioned classes, GL contained a component that had the same TLC mobility as ursolic acid and gave a qualitative reaction with H_2SO_4. Methylation of it gave a product that was identified by TLC as ursolic acid methyl ester. We also previously identified two isomers of triterpene acids, oleanolic and ursolic, during an investigation of glycolipid fractions from seeds of Viburnum [7].

The PL composition and content were determined by the literature micromethod [9]. The amount of PL was $2.2 \cdot 10^{-3}$% of the a-d mass of raw material. Seven components, the main ones of which were PC and PI, were identified in the PL. This agrees with other data reported for the PL composition of this primrose species [3].

Table 1 shows that the FA composition of GL and PL differed from that of NL by being more saturated (due to 16:0 and 18:0 acids). Acids 21:0 and 22:0 were also identified in GL and PL. Linoleic (18:2) acid was the dominant one among unsaturated FA, like in NL. γ-Linolenic acid occurred in significant quantities (7.0% in PL and 5.0% in GL) in the polar lipids.

Thus, the study of lipids from seeds of *O. biennis* introduced in Tatarstan found that they differed insignificantly in lipid and FA composition from those studied earlier [3, 4]. However, they had a lower content of γ-linolenic acid.

EXPERIMENTAL

Mass spectra (EI) were recorded on a MAT-212 mass spectrometer at ionization energy 60 eV and emission current 0.5 mA. Samples were introduced directly into the ion source. The vaporizer temperature was 20°C (initial), gradually heating to 300°C.

Spectrophotometric data were obtained on a Specord M-400 instrument at 800-830 nm (for PL).

GC analysis was carried out on a Chrom-5/DIP chromatograph with a column (1.2 × 3 mm, 5% PDEGS on Chromaton N-AW-DMCS) at 160°C with He carrier gas at flow rate 75 mL/min. Under the chromatography conditions used, γ-linolenic acid had C_{ret} 4.0; α-linolenic, 4.6. Commercially available γ-linolenic acid was used for the identification. α-Linolenic acid was isolated from linseed oil.

Analytical TLC used Silufol plates and glass plates with a deposited layer of LSL 5/40 μm silica gel with 13% gypsum (Chemapol, Czech Rep.).

PL were identified on standard plates (6 × 6 cm) with KSKG silica gel (5-20 μm fraction, 110-150 μm layer thickness, Lyaene Kalur, Khaapsalu) as before [9].

Preparative TLC was performed on glass plates with LSL 5/40 μm silica gel with 13% gypsum (Chemapol, Czech Rep.). NL were desorbed from the silica gel using $CHCl_3$; polar lipids, $CHCl_3$:CH_3OH (2:1). Plates were activated before chromatography by heating in a drying chamber for 60 min at 100°C.

Column chromatography of GL used Silicagel 60 (0.060-0.2 mm, 70-230 mesh, L 14002).

Lipids were separated, purified, and identified using the following solvent systems.

For NL: hexane:diethylether (9:1, 1; 8:2, 2; 7:3, 3; 5:5, 4) and hexane:acetone:benzene:isopropanol (69.5:25.0:4.0:1.5, 5).

For polar lipids: $CHCl_3:CH_3OH:NH_4OH$ (25%) (65:25:4, 6).

For GL: $CHCl_3:(CH_3)_2CO:CH_3OH:CH_3CO_2H:H_2O$ (65:20:10:10:3, 7) and $(CH_3)_2CO:CH_3C_6H_5:CH_3CO_2H:H_2O$ (60:60:20:1, 8).

For PL: $CHCl_3:CH_3OH:C_6H_6:NH_4OH$ (25%) (26:12:4:2.4, 9a) and $CHCl_3:CH_3OH:C_6H_6:(CH_3)_2CO:CH_3CO_2H$ (28:12:4:2:1.6, 9b).

Lipids were developed using iodine vapor for NL; H_2SO_4 solution (50%) with subsequent heating to 120°C until colored spots appeared for triterpene compounds; *o*-phenanthroline solution (0.5%) and $FeCl_3$ solution (0.2%) [10] for tocopherols; α-naphthol and H_2SO_4 (50%) [11] for GL; and Vas′kovsky reagent [9] for PL. Separate PL classes were detected by specific reagents, Dragendorff reagent and ninhydrin solution [11].

Seeds of evening primrose (*O. biennis* L., Onagraceae) from the Kazan′ area were collected for the study.

Oil content, moisture, and acid number were determined by standard methods [12, 13]. NL were isolated from ground seeds by hexane and exhausive soaking at room temperature. Polar lipids were extracted from pulp remaining after NL removal by exhaustive extraction by $CHCl_3:CH_3OH$ (2:1) and soaking at room temperature.

Water-soluble components were removed from the total extract containing total polar lipids by reducing the volume in half in a rotary evaporator and treating with NaCl solution (0.01%).

GL and PL were separated by PTLC in acetone dried over K_2CO_3. GL were separated into classes by CC over silica gel (lipid mass:silica gel mass, 1:50) with elution by $CHCl_3:CH_3OH$ (0-100%).

The quantitative contents of NL and GL were determined gravimetrically in duplicate; PL, by spectrophotometry as described before [9] in triplicate.

Alkaline hydrolysis of acyl-containing lipid fractions was performed in KOH (10%) in CH_3OH (compound:KOH, 1:10) at 60°C for 30 min.

TE were subjected to total alkaline hydrolysis as described before [14]. Methyl esters of FA were prepared by methylation with diazomethane.

Acid hydrolysis of the sterylglycoside fraction was carried out using HCl solution (2 N) at a 2:1 ratio (mg/mL) and refluxing for 5 h. Sterols were extracted by diethylether.

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